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MEMORANDUM:

SUBJECT: **Methyl Parathion** (O,O-dimethyl O-p-nitrophenyl phosphorothioate: Hazard Identification Committee Report.

CASRN: 298-00-0
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FROM: George Z. Ghali, PhD.
Executive Secretary, Hazard Identification Committee
Health Effects Division (7509C)

Thru: Clark Swentzel
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Michael Metzger
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To: Tina Levine, PM 4
Insecticide-Rodenticide Branch
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The Health Effects Division-Hazard Identification Committee met on September 25, 1997 to evaluate the existing and/or recently submitted toxicology data in support of methyl parathion re-registration, identify toxicological endpoints and dose levels of concern appropriate for use in risk assessments for different exposure routes and duration, and assess/reassess the reference dose for this chemical.

Material available for review consisted of data evaluation records (DERs) for combined chronic toxicity-carcinogenicity studies in rats (**83-5**), chronic toxicity studies in dogs (**83-1b**), a carcinogenicity study in mice (**83-2b**), a reproductive toxicity study in rats (**83-4**), developmental toxicity studies in rats and rabbits (**83-3a and -3b**), subchronic studies in rodents and non-rodent species (**82-1a and 82-1b**), a 21-day dermal toxicity study in rabbits (**82-2**), acute and subchronic oral neurotoxicity study

in rats (**81-8ss and 82-**), acute inhalation toxicity studies in rats (**81-3**), and a battery of mutagenicity studies (**84-2**).

INDIVIDUALS IN ATTENDANCE

Hazard Identification Committee members present were David Anderson, Karl Baetcke (Senior Science Advisor, HED), William Burnam (Chief, SAB, HED), George Ghali (Executive Secretary, Hazard Identification Committee, HED), Susan Makris, Nancy McCarroll, Kathleen Raffaele, John Redden, Jess Rowland, Clark Swentzel (Chairman, Hazard Identification Committee, HED).

Others in attendance were William Sette, Diana Locke, Emily Mitchel, William Dykstra, Stephen Dapson and Steven Knizner as observers.

Scientific reviewer(s) (Committee or non-committee member(s) responsible for data presentation; signature(s) indicate technical accuracy of panel report and concurrence with the hazard identification assessment review unless otherwise stated.

Kathleen Raffaele _____

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I. TOXICOLOGY PROFILE:

A. Carcinogenicity:

Three carcinogenicity studies were considered by the committee; two studies in rats and one in mice. One of the two rat studies and the mouse study showed no evidence of increases in tumors. The second rat study showed slight, but not statistically significant, increases in C-cell adenomas in the thyroid, and slight increases in pituitary adenoma, significant in females only. The incidence of pituitary adenomas in females was found to be at the upper end of the historical control range. The Committee concluded, based on the results of statistical analysis, and comparison with historical control data, that these apparent increases were not biologically significant, and do not support a finding that methyl parathion is carcinogenic.

Based on the toxicology data available, the Hazard Identification Committee determined that methyl-parathion did not alter the spontaneous tumor profile in rats and mice under the testing conditions. Therefore, it was recommended that methyl-parathion be classified as a **"Group E"**, indicating evidence of non-carcinogenicity for humans; i.e., the chemical is characterized as **"Not Likely"** to be carcinogenic in humans via relevant routes of exposure. This weight of the evidence judgement is largely based on the absence of significant tumor increases in two adequate carcinogenicity studies in rats (MRID No: 252501-252503) and mice (MRID No. 42216401, 00127239).

This classification is also supported by the lack of mutagenic activity (MRID Nos. 00132949, 00124901, 00124901).

It should be noted, however, that designation of an agent as being in **"Group E"** or **"Not Likely"** is based on the available evidence and should not be interpreted as a definitive conclusion that the agent will not be a carcinogen under any circumstances.

B. Reproductive and Developmental Toxicity:

The following evaluation of the chemical methyl parathion is provided to address FQPA considerations on the sensitivity of infants and children.

1. Reproductive Toxicity:

A two-generation reproduction study was conducted with Sprague-Dawley rats (15 males and 30 females/group) (MRID 00119087;

Doc. 005095, 005588) in which methyl parathion (93.65%) was administered in the diet at levels of 0.5, 5, or 25 ppm (0.04, 0.38, or 2.0 mg/kg/day for males and 0.04, 0.44, or 2.3 mg/kg/day for females). The parental (systemic) NOEL was 5 ppm (0.44 mg/kg/day), and the parental LOEL was 25 ppm (2.3 mg/kg/day), based on decreased prenatally body weight for F1 females and decreased maternal body weight during lactation in females of both generations. No parental reproductive toxicity was observed at any dose level; however, the offspring/developmental NOEL was 5 ppm (0.44 mg/kg/day), based upon decreased pup survival in early lactation and on decreased body weight gain and increased food consumption in the period immediately following weaning. The developmental LOEL was 25 ppm (2.3 mg/kg/day). It was noted that cholinesterase activity was not measured in either adults or offspring in this study.

2. Developmental Toxicity:

In a prenatal developmental toxicity study in Wistar rats (MRID 41136101; Doc. 008118, 009526), doses of 0.3, 1.0, or 3.0 mg/kg/day methyl parathion (97%) were administered by gavage in a dose volume of 10 ml/kg of 0.5% aqueous Cremophor on gestation days 6-15. Each group consisted of 25 rats; 10 additional rats each were assigned to the control and high-dose groups for maternal cholinesterase measurements. Cesarean section was performed on gestation day 21. The maternal NOEL was 1.0 mg/kg/day, with a maternal LOEL of 3.0 mg/kg/day, based upon increased mortality; adverse clinical signs (somnolence, ataxia, dyspnea, ventral recumbency, and repeated chewing behavior); decreased body weight, body weight gain, and food consumption; and decreased plasma, erythrocyte, and brain cholinesterase activity. The developmental NOEL was also 1.0 mg/kg/day; the developmental LOEL (3.0 mg/kg/day) was based on increased postimplantation loss (early resorptions), decreased fetal body weight, and increased incidence of delayed ossification (3rd cervical vertebra, proximal phalanx of the 2nd right digit, and 1st metatarsal of both hindlimbs).

In a prenatal developmental toxicity study conducted in Himalayan rabbits (15/group) (MRID 259403, 259404, 259405; Doc. 004997, 007614), methyl parathion (95.7%) was administered by gavage in 0.5% aqueous Cremophor at dose levels of 0.3, 1.0, or 3.0 mg/kg/day on days 6-18 of gestation. These dose levels were based upon a previously conducted study with rabbits (MRID 41046101) in which plasma and erythrocyte cholinesterase inhibition was observed at a dose of 3.0 mg/kg/day, and for that reason they were considered adequate, although cholinesterase activity was not measured in this study. No evidence of either maternal or developmental toxicity was observed (NOEL \geq 3.0 mg/kg/day).

3. Literature Information:

Although these studies were not submitted to the Agency by the Registrant in support of registration or reregistration, they can be considered in weight-of-the-evidence determinations for methyl parathion.

a. Effects on gestation and morphological development following *in utero* exposure:

In a study by Fuchs et al. (1975), methyl parathion (3 ppm) was administered in the diet to Wistar rats on gestation days (5-9 and 11-15) or (5-9 and 11-19). Growth retardation and increased incidence of resorptions were noted in the treated group, although malformations were not observed.

In a study by Sunil Kamar and Devi (1996), pregnant inbred Wistar rats (10/group) were administered methyl parathion (98% a.i.) on gestation days 6 through 15 at gavage doses of 0.5, 1, and 1.5 mg/kg/day. The dams were killed on gestation day 20 and fetuses were examined for external and visceral anomalies. At 1.5 mg/kg/day, there was a significant decrease in maternal body weight gain during gestation; at the same dose, an increase in resorptions and a decrease in fetal and placental weight were observed. There was no increase in skeletal or viscera abnormalities; however, a significant increase in the incidence of "hemorrhagic spots" in the brain (ventricles) and upper body were observed in pups from dams treated with 1.5 mg/kg/day methyl parathion.

b. Assessment of postnatal functional toxicity following prenatal exposure:

In a study by Gupta et al. (1984), male Fischer 344 rats were mated to Wistar-Furth females. The dams were administered 1.0 mg/kg/day of methyl parathion in peanut butter (0.1 g/25 g body weight) as a dietary dose consumed in <2 minutes, or 1.5 mg/kg/day of methyl parathion by gavage in peanut oil (at a volume of 0.1 ml/50g body weight). Treatment was administered daily from gestation day 6 through 20. The dams were allowed to litter normally, and pups were placed with foster mothers within 24 hours of birth. Pups and dams were killed at intervals (postnatal days 1, 7, 14, 21, and 28 for pups, and at gestation day 19 for dams). Their brains were removed, weighed, dissected, and processed for analysis of acetylcholinesterase (Ache) and choline acetyltransferase (CAT) activity and of [³H]quinuclidinyl benzilate (³H-QNB) binding to muscarinic receptors. Frontal cortex and brainstem were collected on postnatal days 1 and 7, while striatum and hippocampus were also obtained on postnatal days 14,

21, and 28. Tissues from two pups per litter were pooled, and the litter was used as the unit of analysis. Behavioral evaluation of the pups was performed: preweaning reflexive behaviors (postnatal days 1-25); startle response (days 1-25 and 4 months); passive avoidance, rotarod performance, and accommodated locomotor activity (2 months); cage emergence (3 months); shuttle box avoidance (4 months); and operant behavior (3-6 months). Morphological analysis of the cornuammonis in the hippocampus and of the cerebellar culmen was performed in 4 control and 4 high-dose pups at 28 days of age.

The following treatment-related effects were noted: At 1.5 mg/kg/day, clinical signs of toxicity included muscle fasciculations and tremors, decreased maternal body weight gain and increased late resorptions. On postnatal day 1, litter size, body weight, and pup brain weight were similar between control and treated groups. Prenatal exposure to 1.5 mg/kg/day reduced Ache and increased CAT activity in all brain regions at each developmental period and in maternal brain. Similar exposure to 1.0 mg/kg/day caused a significant but smaller and less persistent reduction in Ache activity but no change in brain CAT activity of the offspring. Both dose levels decreased the B_{\max} of ^3H -QNB binding in maternal frontal cortex but did not alter the postnatal pattern of ^3H -QNB binding. Cage emergence, accommodated locomotor activity, and operant behavior in a mixed paradigm were impaired in rats exposed to 1.0 but not to 1.5 mg/kg/day. The study authors concluded that "subchronic prenatal exposure to methyl parathion altered postnatal development of cholinergic neurons and caused subtle alterations in selected behaviors of the offspring."

c. Comparison of the neurotoxic response of adults and neonatal or weanling animals:

In a study by Benke and Murphy (1974), the effects of methyl parathion and methyl paraoxon were studied in male and female Holtzman rats ranging in age as follows: 1, 12-13, 23-24, 35-40, and 56-63 days of age. The test substances were administered by i.p. injection in corn oil at a volume of 1 ml/kg over a range of doses. It was found that there was a gradual decrease in susceptibility to methyl parathion with increasing age for both sexes as measured by the value of the LD50. For methyl parathion, the LD50 ranges from 1 mg/kg at postnatal day 1 to 6-8 mg/kg on postnatal day 56-63. Age differences in susceptibility were not related to differences in sensitivity of cholinesterase to inhibition by methyl paraoxon *in vitro*. LD50 values were calculated for the different ages; in general, changes in LD50 values with age for methyl parathion correlated better with changes in rates of reactions which represented detoxification pathways for methyl paraoxon than for reactions which represented direct

metabolism of the parent compound. Both male and female rats became less sensitive to the acute lethal effects of methyl paraoxon with increasing age. This is consistent with a hypothesis that changes in LD50 values of methyl parathion with age are due to changes in rates of metabolism of the oxygen analogs.

In a study by Pope et al. (1991), the time course of cholinesterase inhibition and recovery in whole brain was compared between neonatal (postnatal day 7) and adult (80-100 days of age) Sprague-Dawley rats after acute treatment (by subcutaneous injection) with maximum tolerated doses of methyl parathion and other organophosphate pesticides (chlorpyrifos and parathion). The neonates were more sensitive clinically than adults to chlorpyrifos exposure: the MTD for neonates was 7.8 mg/kg s.c., while for adults the MTD was 18 mg/kg s.c. In general, maximal brain ChE inhibition was similar (>78%) in both age groups, but ChE activity recovered faster in neonates. Plasma and RBC ChE activities correlated relatively well with brain ChE activity in neonatal rats at all time points between 4 hours and 7 days posttreatment, but similar correlations between circulating and brain ChE activities in adults were more variable. The study authors concluded that neonatal rats are more sensitive to acute lethality from methyl parathion (and other OP) exposure than are adults, and that MTD exposures produced extensive brain ChE inhibition in both age groups. Following OP exposures, however, significant compound-related and age-related differences in the duration of ChE inhibition can occur.

In a study by Pope and Chakraborti (1992), dose-related inhibition of both brain and plasma cholinesterase activity was examined in neonatal and adult rats exposed to methyl parathion and other organophosphate pesticides (chlorpyrifos and parathion) by subcutaneous injection in corn oil at 1-2 ml/kg. It was found that ED₅₀ estimates for both brain and plasma cholinesterase correlated highly with previously derived MTD values. The correlation between the extent of brain and plasma cholinesterase inhibition across dose in neonatal rats was high but lower in adults. The study authors concluded that in vivo inhibitory potency, towards either brain or plasma ChE activity, of methyl parathion and the other organophosphate pesticides tested, is highly correlated with sensitivity to acute toxicity in both neonatal and adult rats.

4. Developmental Neurotoxicity:

There was no developmental neurotoxicity study available for review by the Committee. The Committee determined that a developmental neurotoxicity study should be required for methyl parathion. It was further recommended that the protocol should include comparative measurements of cholinesterase inhibition in adults and offspring. The weight-of-evidence used in arriving at

this conclusion is presented below:

- a. Evidence that support requiring a developmental neurotoxicity study:

Methyl parathion is a neurotoxic chemical (methyl parathion is an organophosphate compound, administration of methyl parathion to various species including rat, mouse, dog, and rabbit results in plasma, RBCs, and brain cholinesterase inhibition sometimes accompanied by cholinergic symptoms (e.g., lacrimation, salivation, miosis, tremors, convulsions, muscle fasciculation, muscle weakness, ataxia) as observed in rats in an acute neurotoxicity study at a gavage dose of 7.5 mg/kg and other cholinergic symptoms (e.g., tremors, slow pupillary constriction, and decreased hindlimb grip strength were observed at 50 ppm (3.02/3.96 mg/kg/day in M/F) in the subchronic neurotoxicity study.

Neuropathological findings observed in the acute neurotoxicity study in rats (at 7.5 mg/kg) included focal demyelination of the dorsal and ventral root fibers of the cervical and lumbar spinal cord and focal demyelination of the sural and tibial nerves. In the subchronic neurotoxicity study, the incidences of degenerative lesions of peripheral nerves at 50 ppm (3.02/3.96 mg/kg/day in M/F) were equivocal. In the two-year chronic study in Sprague-Dawley rats, loss of myelinated sciatic nerve fibers and retinal atrophy were observed at 50 ppm (2.5 mg/kg/day).

There is evidence of the developmental neurotoxic potential of methyl parathion in the open literature. In a study by Gupta, et al. (1985), it was demonstrated that both maternal and fetal neurobiochemical markers are affected by treatment with 1.0 or 1.5 mg/kg/day from gestation days 6-20, and that altered postnatal development of cholinergic neurons and alteration of select behaviors of the offspring resulted.

Methyl parathion is extremely toxic on acute basis; the oral LD50 in rats is approximately 4.0 mg/kg (males) and 6.3 mg/kg (females).

- b. Evidence that do not support asking for a developmental neurotoxicity study:

Brain weight was increased in the three-month study in mice at 60 ppm (13.5/16.2 mg/kg/day in M/F) and in the two-year chronic study in rats at 50 ppm (2.5 mg/kg/day). These effects were, however, not statistically significant and were not considered to be biologically meaningful. In a study from the open literature (Gupta et al., 1985) it was reported that pup brain weights (Day 1)

were not affected following *in utero* exposure to methyl parathion (gestation days 6-20). Furthermore, delayed neuropathy was not observed in the hen.

No evidence of abnormalities in the development of the fetal nervous system was observed in the prenatal developmental toxicity studies in either rats, or rabbits, at maternal gavage doses up to 3.0 mg/kg/day. In the two-generation reproduction study in rats, no clinical evidence suggestive of neurotoxicity was observed grossly in pups, which had been administered methyl parathion *in utero* and during early and late postnatal development, generally mediated by maternal dietary exposure, but also available in the diet to late lactation pups.

There is no evidence that endocrine function is disrupted by administration of methyl parathion.

C. FQPA Considerations:

Under the Food Quality Protection Act (FQPA), P.L. 104-170, which was promulgated in 1996 as an amendment to the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug and Cosmetic Act (FFDCA), the Agency was directed to "ensure that there is a reasonable certainty that no harm will result to infants and children" from aggregate exposure to a pesticide chemical residue. The law further states that in the case of threshold effects, for purposes of providing this reasonable certainty of no harm, "an additional tenfold margin of safety for the pesticide chemical residue and other sources of exposure shall be applied for infants and children to take into account potential pre- and post-natal toxicity and completeness of the data with respect to exposure and toxicity to infants and children. Notwithstanding such requirement for an additional margin of safety, the Administrator may use a different margin of safety for the pesticide residue only if, on the basis of reliable data, such margin will be safe for infants and children."

Pursuant to the language and intent of the FQPA directive regarding infants and children, the applicable toxicity database for methyl parathion was evaluated by the Hazard Identification Committee.

Adequacy of data package: The data package included an acceptable two-generation reproduction study in rats and acceptable prenatal developmental toxicity studies in rats and rabbits, meeting the basic data requirements, as defined for a food-use

chemical by 40 CFR Part 158. A developmental neurotoxicity study was requested.

Susceptibility issues: The submitted guideline study data provided no indication of increased sensitivity of rats or rabbits to *in utero* and/or postnatal exposure to methyl parathion. In the two-generation reproduction study in rats, offspring toxicity occurred at the same dose as parental toxicity; the offspring developmental and parental systemic NOELs and LOELs were 5 ppm (0.25 mg/kg/day) and 25 ppm (1.25 mg/kg/day), respectively. In the prenatal developmental toxicity study in rats, developmental toxicity was observed only in the presence of maternal toxicity; the maternal and developmental NOELs and LOELs were equivalent at 1.0 and 3.0 mg/kg/day, respectively. In the prenatal developmental toxicity study in rabbits, neither maternal nor developmental toxicity was observed, although, based upon the results of a previous study in rabbits, the doses were judged to be adequate to elicit plasma and erythrocyte cholinesterase inhibition in the maternal animals.

An assessment of the differential response of fetuses versus adults to cholinesterase inhibition following oral administration of methyl parathion was studied by Gupta, et al. (1985). No indication of additional sensitivity of the offspring was suggested by the data, since offspring effects were noted concurrently with maternal effects. Specifically, it was demonstrated that both maternal and fetal neurobiochemical markers are affected by treatment with 1.0 or 1.5 mg/kg/day from gestation days 6-20, and that altered postnatal development of cholinergic neurons and alteration of select behaviors of the offspring resulted.

In addition, in studies by Benke and Murphy (1974), Pope et al. (1991), and Pope and Chakraborti (1992), evidence of increased sensitivity of young rats to the effects of methyl parathion, as compared to adults, were reported. Although these studies used non-oral methods of test substance administration, and were conducted at the maximum tolerated dose in order to establish LD50 values, they indicate that there should be a concern for the effects of methyl parathion on young animals.

Uncertainty factor: Based on the following concerns, the Committee determined that for methyl parathion the 10-fold uncertainty factor for the protection of infants and children is appropriate:

1. The data base for methyl parathion is complete with regard to the standard studies required by 40 CFR Part 158 for a food-use chemical. Acceptable prenatal developmental toxicity

studies in rats and rabbits, and an acceptable multigeneration reproduction study in rats have been received by the Agency. Although delayed neuropathy was not observed in a study in hens, a single-dose acute neurotoxicity study in rats demonstrated neuropathology at a relatively low dose level (2.5 mg/kg). Also, there was evidence of the developmental neurotoxic potential of methyl parathion in the open literature (Gupta et al., 1985); in this study, altered postnatal development of cholinergic neurons and alteration of select behaviors of the offspring resulted following *in utero* exposure. A developmental neurotoxicity study is required with methyl parathion; in the absence of this study, substantial uncertainties remain regarding the effect of methyl parathion on functional development.

2. Although differential sensitivity to young animals was not revealed in standard prenatal developmental and multigeneration reproductive toxicity studies, qualitative evidence of increased sensitivity to perinatal rats has been identified in the open literature. In these studies, a) lethality at doses at or near the maximum tolerated dose and b) cholinesterase inhibition were used as biomarkers of sensitivity. Methyl parathion was administered to the rats in these studies by subcutaneous injection (Pope et al., 1991; Pope and Chakraborti, 1992), intraperitoneal injection (Benke and Murphy, 1974), or oral (Gupta, 1984) routes. This evidence of increased sensitivity to the offspring cannot be quantified, however data from another organophosphorus pesticide, chlorpyrifos, has demonstrated differences in sensitivity in the offspring of up to 10-fold.

D. Mutagenicity:

Seven studies sponsored by the USEPA under contract Nos. 68-02-2947 or EPA-600/1-7-028 were available for review. The following are summaries and the committee's conclusion for these studies (with MRID/Accession and/or Document Control Numbers):

1. Gene mutations:

a) Salmonella typhimurium reverse gene mutation assay (MRID No. 00124901; Doc. No. 005095): The test is negative in S. typhimurium strains TA1535, TA1537, TA1538 and TA100 up to 1000 µg/plate +/-S9, the highest dose tested (HDT). Due to the lack of cytotoxicity at the HDT and other technical deficiencies, the study was classified as Unacceptable. However, the standard protocol used in this assay required testing up to 10,000 µg/plate +/-S9, unless solubility or cytotoxic prevent testing up to this level. The Committee, therefore, concluded that methyl parathion was assayed up to an appropriate high dose and found to be negative.

It was further concluded that the assay should be upgraded.

b) Escherichia coli reverse gene mutation assay (MRID No. 00124901; Doc. No. 005095): The test is negative in E. coli WP2 up to the HDT (1000 µg/plate +/-S9). Due to the lack of cytotoxicity at the HDT and other technical deficiencies, the study was classified as Unacceptable. For reasons similar to those stated above, the Committee concluded that the assay should be upgraded.

c) Saccharomyces cerevisiae D7 reverse gene mutation, mitotic gene conversion and mitotic crossing-over assay (MRID No. 00132949; Doc. No. 005095): Independent test are negative at all three endpoints up to severely cytotoxic levels ($\geq 1.0\%$ +/-S9, equivalent to $\approx 10,000$ µg/mL).

d) L5178Y TK $^{+/-}$ mouse lymphoma cell forward gene mutation assay (MRID No. ?; Doc. No. 005095/005588): Confirmed positive; dose-related mutagenic effects at 80-200 µg/mL with S9 activation. Positive responses were also seen in the absence of S9 activation; however, the effect was not clearly dose-related and reproducible increases in the mutation frequency were only seen at ≥ 150 µg/mL. (Colony sizing was not performed).

2. Chromosomal Aberrations:

a) Mouse Dominant lethal assay (MRID No. 00124901; Doc. Nos. 005095/005588): The test is negative in the germinal cells of male ICR/SIM mice receiving dietary administrations of 0, 20, 40 or 80 ppm methyl parathion for 7 weeks. No overt toxicity or cytotoxicity to the target organ occurred at the HDT. It was noted, however, that body weight reductions (4-20%) were seen at all weeks in males mice receiving dietary levels of 10, 30 or 60 ppm methyl parathion in the subchronic mouse study conducted with methyl parathion (MRID No. 00072513). Based on these findings, the Committee concluded that dosing was adequate in the dominant lethal assay.

3. Other Mutagenic Mechanisms:

a) In vitro sister chromatid exchange (SCE) in Chinese hamster ovary cell assay (MRID No. ?; Doc. No. ?): The test is positive in the presence of S9 activation; dose related increases in SCEs were obtained at 50-200 µg/mL. Without S9 activation the test is negative up to cytotoxic levels (≥ 40 µg/mL).

b) Unscheduled DNA synthesis in cultured human fibroblasts (WI-38) assay (MRID No. 00124901; Doc. No. 005095/005588): The test is negative up to a precipitating dose (10^{-3} M +/- S9).

4. Conclusions:

Methyl parathion was negative for gene mutations in S. typhimurium, E. coli and S. cerevisiae. It also did not cause mitotic recombination or gene conversion in S. cerevisiae or DNA damage in a human cell line. Gene mutations and SCE were, however, induced in cultured mammalian cells and the effect was more clearly demonstrated in the presence of S9 activation. The only acceptable in vivo study in the Agency's files indicated that methyl parathion was not active in the mouse dominant lethal assay. Nevertheless, positive dose-related increases in micronuclei induction have been reported in the literature in mice receiving methyl parathion orally (Mathew et al., 1990) and in rats following intraperitoneal injection (Grover and Malhi, 1985). Structural chromosome aberrations have also been reported in bone marrow cells harvested from treated rats (Malhi and Grover 1987).

The relevance of the positive findings from both the in vitro and in vivo mutagenicity studies is not clear in light of the negative cancer studies and the lack of an effect in germinal cells in the dominant lethal assay. The Committee concluded, therefore, that nothing further would be gained by requiring additional testing. Based on these deliberations, the available acceptable studies satisfies the pre-1991 mutagenicity initial testing battery guidelines. No further testing is required at this time.

E. Dermal Absorption:

No dermal absorption study was available. Because of relatively high dermal toxicity, dermal absorption should be assumed to be 100%.

II. HAZARD IDENTIFICATION:

Based on comprehensive evaluation of the toxicology data available on methyl parathion, toxicology endpoints and dose levels of concern have been identified for use in risk assessments corresponding to the categories indicated below.

Where no appropriate data have been identified for a particular duration or exposure scenario, or if a risk assessment is not warranted, this is noted. Levels of uncertainties associated with intraspecies variability, interspecies extrapolation, route to route conversion, or variable durations extrapolation are also addressed.

Based on the exposure/use profile for methyl parathion, the Committee determined that the risk assessments indicated below are required.

A. Chronic Dietary Exposure-Reference Dose (RfD):

Reference Dose (R_fD): 0.00002 mg/kg/day mg/kg/day.

Critical Study: A Two Year Chronic Feeding Study of Methyl Parathion in Rats (83-1a), MRID No 252501-252503.

Executive Summary: Methyl Parathion (purity 93.7%) was administered to Sprague Dawley rats (60/sex/group) at 0, 0.5, 5, and 50 ppm in the diet (mean compound intake approximately 0, 0.02, 0.21, and 2.21 mg/kg/day for males and 0, 0.03, 0.29, and 3.34 for females [from the original study report, based on nominal concentrations]) for 26 (males) or 28 (females) months. Body weights and food consumption were recorded weekly to week 14, and biweekly thereafter. Ophthalmic examinations were performed at pretest, 3, 12, and 24 months and on females only at 28 months. Clinical chemistry and hematological parameters were determined, and urinalysis was performed at 6, 12, 18, and 24 months (10 animals/sex/group). Plasma and RBC Cholinesterase inhibition were also measured at 6, 12, and 18 months and at termination (10 rats/sex/group), on different animals from those used for other blood measures. Brain cholinesterase was measured at termination. All study animals received gross postmortem examination and histopathological evaluations. Five animals/sex/dose received additional histopathological examination of neural tissues (brain, spinal cord, and sciatic nerve).

No carcinogenic effects were seen at 50 ppm (highest dose) in either sex. Doses were considered adequate to test for carcinogenicity.

Effects seen at the 5.0 ppm dose were abnormal gait in one female, significant decreases in hematocrit and erythrocyte levels in males at 24 months, slight decreases in erythrocyte cholinesterase activity in both sexes (+4.4 to -11.3% inhibition).

Additional effects seen at 50 ppm were significant decreases in hemoglobin, hematocrit, and erythrocyte levels in females (at all time points), significant decreases in activity of plasma (67-89% inhibition), erythrocyte (0-20% inhibition), and brain cholinesterase (76-79% inhibition) at multiple time points (males and females), increased incidence of alopecia (more pronounced in females), bilateral retinal degeneration and posterior subcapsular cataract (females only, at 24 months), decreased mean body weight and increased food consumption (both sexes), irritability (both sexes), tremors (largely in females), increased incidence of anogenital staining, decreased incidence of chromodacryorrhea, and soft stools. There was also a slight apparent increase in survival in 50 ppm females.

Neurological changes (in particular sciatic nerve degeneration) were most pronounced in animals receiving 50 ppm, but lesions in low and mid-dose males were slightly more severe than those seen in control animals. Although the original DER found no NOEL for these effects, upon re-evaluation a NOEL can be determined at the low dose (0.5 ppm).

The LOEL for neurological effects in this study, based on increases in lesion severity (sciatic nerve degeneration, males) was established at 5.0 ppm (0.21 mg/kg/day in males, 0.29 mg/kg/day in females); the NOEL was established at 0.5 ppm (0.02 mg/kg/day for males, 0.03 mg/kg/day for females). Based on other effects seen in this study (decreased hematocrit and erythrocyte levels), the systemic LOEL was established at 5.0 ppm (0.21 mg/kg/day in males, 0.29 mg/kg/day in females); the NOEL was established at 0.5 ppm (0.02 mg/kg/day in males, 0.03 mg/kg/day in females). The LOEL for cholinesterase inhibition (decreased erythrocyte cholinesterase activity in both sexes) was established at 5.0 ppm (0.21 mg/kg/day in males, 0.29 mg/kg/day in females); the NOEL was established at 0.5 ppm (0.02 mg/kg/day in males, 0.03 mg/kg/day in females).

This study is classified as Acceptable for carcinogenicity and Acceptable for chronic toxicity in rats.

Endpoint and Dose selected for use in risk assessment: 0.02 mg/kg/day was the NOEL for this study, based on systemic toxicity, neuropathology, and RBC cholinesterase inhibition occurring at 0.21 mg/kg/day.

Uncertainty Factor (UF): An uncertainty factor of 100 was applied to account for both interspecies extrapolation and intraspecies variability. The use of a UF of 100 was justified based on the availability of a chronic toxicity study in a second species (MRID Nos.) and a reproductive toxicity study in rats (MRID No. 00119087) in accordance with the rules established by the Agency-Integration Risk Information System (IRIS) Work Group.

The Committee recommended that the additional UF of 10, required for the protection of infants and children in accordance with the FQPA, be retained in addition to the traditional Uncertainty Factor (see Section I-C, above).

Comments: This study is appropriate for use in risk assessment for this type of exposure and duration concern.

B. Acute Dietary Exposure (one day):

Critical Study: Acute Neurotoxicity (81-8), MRID No.: 43254401.

Executive Summary: In this acute neurotoxicity study, male and female Sprague-Dawley rats (10 animals/sex/group) were orally gavaged once with methyl parathion at doses of 0, 0.025, 7.5, 10 (males only), or 15 (females only) mg/kg. Neurobehavioral evaluations, consisting of FOB and motor activity, were conducted at pre-study, at the peak time of effect (1.5 hrs post-dosing) on Day 0 and on Days 7 and 14. At 15 \pm 3 days animals were euthanized and neuropathological examination performed on control and high-dose animals (6/dose/sex). Plasma and erythrocyte (RBC) cholinesterase activities were determined at Day -2; plasma, RBC and brain (six different regions) activities were measured at the peak time of effect and at Day 14.

No significant differences were noted in the mean body weights of the treated animals; body weight gain in high-dose males was significantly lower than controls.

Neurobehavioral evaluation revealed treatment-related FOB and motor activity findings at the mid- and high-dose levels. The effects were transient and observed only at the peak time of effect. Neurobehavioral findings are consistent with those observed following cholinesterase inhibition (i.e. lacrimation, salivation, miosis, tremors/convulsions, muscle fasciculations, muscle weakness, and ataxia).

No treatment-related gross pathological findings were observed. Neuropathological findings consisted of focal

demyelination in the dorsal root fibers of the cervical spine in 3/6 high-dose males and lumbar spine in 3/6 low-, 4/6 mid- and 5/6 high-dose males. Focal demyelination was also observed in the ventral root fibers of the cervical spine in 2/6 high-dose males and of the lumbar spine in control (males, 2/6; females, 1/6), low- (males, 3/6), mid- (males, 4/6), and high- (males, 4/6; females, 3/6) dose groups. Focal demyelination of the lumbar spinal cord and spinal nerve were observed in high-dose males; the incidence of each of these observations was only 1/6. Focal demyelination was observed in the tibial nerves of 1/6 mid- and 3/6 high-dose males and in the sural nerves of 2/6 high-dose males.

In summary, systemic toxicity was observed in high-dose males (decreased body weight gain) and females (increased incidence of clinical signs). Neurotoxic effects (abnormal FOB findings, decreased motor activity, inhibition cholinesterase activities, and neuro-pathological findings) were observed in mid- and high-dose males and females.

Based on the results of this study, the systemic LOEL was 10 mg/kg (males) and 15 mg/kg (females); the systemic NOEL was 7.5 mg/kg. In males and females, the LOEL for neurotoxicity was 7.5 mg/kg; the NOEL for neurotoxicity was 0.025 mg/kg.

This study is classified as Core-Guideline and satisfies guideline requirements (81-8) for an acute neurotoxicity screening battery in the rat.

Endpoint and Dose Selected for Use in Risk Assessment: 0.025 mg/kg was the NOEL for this study, based on neurotoxicity and cholinesterase inhibition occurring at 7.5 mg/kg.

Comments and Rationale: Effects seen in this study occurred after a single dose, appropriate for use in risk assessment for this type of exposure and duration concern.

Uncertainty Factor (UF): The Committee recommended that the additional UF of 10, required for the protection of infants and children in accordance with the FQPA, be retained in addition to the traditional Uncertainty Factor.

C. Short Term Occupational or Residential Exposure (1-7 days):

Critical Study: Acute Neurotoxicity (81-8), MRID No.: 43254401.

Executive Summary: See Section II-B, above.

Endpoint and Dose Level selected for use in risk assessment: Same as for acute oral, 0.025 mg/kg, based on neurotoxicity and cholinesterase inhibition occurring at 7.5 mg/kg.

Comments: Although there was a 21-day dermal study in rabbit available, it was not selected for the following reasons: 1) The rabbit is less sensitive than the rat to this chemical (for example, in the rabbit developmental study, 3.0 mg/kg doses resulted in only minimally significant cholinesterase inhibition); 2) several endpoints (including neurotoxicity and neuropathology) occurring at low doses in the acute oral study were not measured in the dermal study; 3) oral and dermal effects seen in other acute studies occurred at similar doses (see Acute Toxicity Endpoints), so there is no reason to believe that neurotoxic effects might not occur at low dermal doses, and 4) because of physiological and biochemical factors, unique to the rabbit, which might result in underestimation of the dermal toxicity of organophosphorus pesticides belonging to the thiophosphate subgroup (R. Zendzian, HED, memo dated March 1997). Dermal absorption rate was assumed to be 100%.

Uncertainty Factor (UF): The Committee recommended that an additional UF of 10 be applied in addition to the traditional Uncertainty Factor (see Section I-C, above).

D. Intermediate Term Occupational or Residential Exposure (one week to several months):

Critical Study: A Two Year Chronic Feeding Study of Methyl Parathion in Rats (83-1a), MRID No 252501-252503.

Executive Summary: See Section II-A, above.

Endpoint and Dose Level Selected for Use in Risk Assessment: 0.02 mg/kg/day was the NOEL for this study, based on systemic toxicity, neuropathology, and RBC cholinesterase inhibition occurring at 0.21 mg/kg/day.

Comments: Long term dermal study is not available. Since there is evidence of cumulative neurotoxic effects, the acute neurotoxicity study is not of sufficient duration to cover this type of exposure. Dermal absorption rate was assumed to be 100%.

Uncertainty Factor (UF): The Committee recommended that the an additional UF of 10 be applied in addition to the traditional Uncertainty Factor (see Section I-C, above).

E. Chronic Occupational or Residential Exposure (several

months to life time):

Critical Study: A Two Year Chronic Feeding Study of Methyl Parathion in Rats (83-1a), MRID No 252501-252503.

Executive Summary: See Section II-A, above.

Endpoint and Dose Selected for Use in Risk Assessment: 0.02 mg/kg/day was the NOEL for this study, based on systemic toxicity, neuropathology, and RBC cholinesterase inhibition occurring at 0.21 mg/kg/day.

Uncertainty Factor (UF): The Committee recommended that the additional UF of 10 be applied in addition to the traditional Uncertainty Factor (see Section I-C, above).

Comments: Long term dermal study is not available. Therefore, a chronic oral toxicity study was used and a 100% dermal absorption rate was recommended.

F. Inhalation Exposure (variable duration):

** Use the same format as the dermal if appropriate studies are available for the 3 exposure time periods. If appropriate studies are NOT available for the 3 time periods then use the following format.*

Critical Study: A Two Year Chronic Feeding Study of Methyl Parathion in Rats (83-1a), MRID No 252501-252503.

Executive Summary: See Section II-A, above.

Endpoint and Dose Level Selected for Use in Risk Assessment: 0.02 mg/kg/day was the NOEL for this study, based on systemic toxicity, neuropathology, and RBC cholinesterase inhibition occurring at 0.21 mg/kg/day.

Comments: Due to high toxicity seen in acute inhalation study, 100% absorption should be assumed.

Uncertainty Factor (UF): The Committee recommended that the additional UF of 10, required for the protection of infants and children in accordance with the FQPA, be retained in addition to the traditional Uncertainty Factor (see Section II-A, above).

G. Aggregate Risk:

The Comittee recomended that the aggregate risk be performed by adding the exposures together and using the oral endpoint. As previously stated, the dermal absorption rate was assumed to be 100%.

III. APPENDIX:

A. Acute Toxicity:

Guideline No.	Study Type	MRID #(s).	Results	Toxicity Category
81-1	Acute Oral (rat)		LD ₅₀ = 4.5-24 mg/kg	I
81-2	Acute Dermal (rat)		LD ₅₀ = 6 mg/kg	I
81-3	Acute Inhalation (rat)	256961	LC ₅₀ <0.163 mg/L	I
81-4	Primary Eye Irritation	256966, 40542602	Irritation clear by 7 days	III
81-5	Primary Skin Irritation	256962	Max. score=2.0; 72 h=0.5	IV
81-6	Dermal Sensitization	256963	Negative	
81-8	Acute Neurotoxicity Delayed Hen	41606801	Negative	--

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